SUPRAVITAL STAINED WET MOUNT STUDY OF FINE NEEDLE ASPIRATES - AS A RAPID SUPPLEMENTARY DIAGNOSTIC PROCEDURE

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CERTIFICATE

This is certified that this dissertation titled **"Supravital stained wet mount study of fine needle aspirates – As a rapid supplementary diagnostic procedure"** is the bonafide work of **Dr. S. Sumathi** who carried out the work under my guidance **Dr. V. Paramasivan, M.D.**, Professor and Head, Department of Pathology Tirunelveli Medical College, Tirunelveli.

Professor and Head of the Department

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CONTENTS

Introduction

Fine needle aspiration cytology (FNAC) technique has become more common and popular nowadays. Though it is not a substitute for conventional histopathology, it should be regarded as a very important component of pre operative / pre treatment investigation in combination with clinical, radiological and other laboratory data.

Conventional FNAC has its own advantages and limitations, which are well known to any cytopathologist. Inspite of its advances and advantages, conventional FNAC fails to achieve a 100% accuracy.

This is partly because of,

(i) A lack of sufficient cellularity in desmoplastic lesions.

(ii) Wastage of aspirated cells when they stick to the hub and lumen of the needles.

(iii) Morphological distortion produced when the cells are trapped in fibrin mesh.

(iv) Distortion of fragile cells during smearing.

(v) Loss of cell to cell and cell to stromal histological architecture.

Hence in an attempt to improve it's accuracy, a supra vital stained wet mount preparation of the aspirate is studied which will give additional information regarding the lesion on which FNAC is done. Here the same needle and syringe which is used to aspirate for conventional fixed smear (H & E) is immediately dipped into toludine blue solution and rinsed. The mixed material is poured on a clean slide along with a drop of 0.5% eosin and cover slip is placed over it. Then this wet mount is examined under microscope for morphological details.

Toludine blue is a rapid supravital stain that produce good nuclear detail and giving a three dimensional view of cell in a wet mount. It is also easily available and inexpensive. Addition of eosin stain gives a pale pink cytoplasm to the cell and gives better contrast to appreciate cytomorphology of wet mount well. Supravital stained wet mount of fine needle aspirate is becoming popular as a supplementary procedure to conventional FNAC smears.

Aims

This study is aimed at,

(i) Studying wet mount preparation of materials obtained by FNAC, guided FNAC, body cavity fluids after supravital staining with toludine blue/eosin.

(ii) Comparing the three dimensional morphological patterns in rapid staining technique with conventional H & E stained smears.

(iii) Assessing the reliability and accuracy of toludine blue / eosin stained wet mount preparation study, as compared to conventional FNAC, histopathological and clinical evaluation.

(iv) Assessing whether it could help in improving the diagnostic accuracy of conventional FNAC by simultaneously doing supravital wet mount as a supplementary procedure.

Review of literature

For over 100 years, the discipline of anatomical pathology has centered on diagnostic histopathology and this in turn on the surgical biopsy. For the last 60 years, exfoliated and abraded samples of cells have also been collected from accessible anatomical surfaces especially from uterine cervix and the bronchus. In the United Kingdom, Dudgeon and Patrick (1927) proposed the needling of tumors as a means of rapid microscopic diagnosis. Consequently, Martin and Ellis (1930) used needles of thicker calibre for aspiration biopsy than those commonly in use today. It was in Europe and particularly Scandinavia the fine needles aspiration cytology began to flourish in the 1950s and 1960s.

Fine needle aspiration cytology became more popular and common now. Initially the method was applicable to lesions that are easily palpable-superficial growth of the skin, subcutis and soft tissues and organs such as thyroid, breast, salivary glands and superficial lymph nodes. Modern imaging techniques mainly ultrasonography and computed tomography applied to organs and lesions in sites not easily accessible to surgical biopsy offer vast opportunities for fine needle aspirations of deeper structures. So sample may be obtained from the lung, mediastinum, abdominal, retroperitoneal and pelvic organs and, deep seated lesions in head and neck and soft tissues.

Fine needle aspiration cytology (FNAC)

Needles

The size of the needles used for aspirating materials are varied according to the site of lesion. Standard disposable 27-22 G, 30-50 mm needles are suitable for superficial palpable lesions, 22 G, 90 mm disposable lumbar puncture needles with trocar are convenient for most deep biopsies. There have been some variations in needle design as aspiration biopsy has become more widely used. The Franzen needle used for prostatic FNAC has a notched tip and stylus (William J. Frable). The Milex and Inrad needles that were designed to improve sampling in firm fibrous breast masses have a slot on the side, so called side port needles. Radiologists use the Chiba needle of 21-22 G most frequently for transthoracic and transabdominal aspirations. In some centers, bone lesions are also aspirated and combined with core biopsy (Layfield *et al*., 1987 and White *et al*., 1988). There are virtually no complications by employing only the thin needle technique except for pneumothorax with thransthoracic aspiration and some cases of excessive bleeding with transabdominal aspiration (Powers *et al*., 1995).

Technique

The skin should be cleansed with an alcohol swab prior to puncture for superficial FNAC. Local anesthesia is used for percutaneous radiologically guided needle biopsies. The sterile needle is inserted in the target area. Staying within the lesion, the needle is moved in a cutting motion, withdrawing cells into the needle hub. The force of the cutting motion needed to obtain an adequate sample must be adjusted for the body site and characteristic of the lesion. These biopsies may be performed with "Suction" (Aspiration) or "Non Suction" (Non aspiration) technique. Once the cellular material is seen in the needle hub, suction is released and needle is withdrawn. The cellular material is expressed on to one or more slides and is smeared with a second slide. One of the slide is fixed in a fixative and the other is air dried.

Fixatives

For routine wet fixation of smear either 70-90% alcohol in Coplin jar or commercial spray fixatives are used. Carnoys fixative has the advantage of lysing red blood cells and being a good nuclear fixative.

Sampling of fluids

In case of cystic lesions, as much fluid as possible is removed. Then the cyst fluid can be handled as liquid specimen. If there is a residual mass, the procedure for solid lesions as described above should be followed.

Serous or body cavity fluids are usually aspirated with aseptic technique by a needle puncture and fluid is collected in a dry container. Fluid sample is submitted in fresh state to the laboratory. If delay in transportation to the laboratory is unavoidable, fluid may be kept in a refrigerator at 4^{o} C upto 6 hrs. Fluid samples intended for cytological analysis should never be frozen. If longer delay is expected, preservation at the time of collection with 50% ethanol equal to the volume of specimen is suggested. For small fluid accumulations the entire specimen is submitted for laboratory evaluation. For large effusions, 50-200 ml of well mixed fluid should be sent for cytological examination. Fluid received is centrifuged at 1500 rpm for 10 minutes. The supernatant decanted and smears are made from upper layer of sediment. If the specimen is very bloody, variable amount of Carnoys solution depending on the amount of red blood cell present can be added, mixed, left to stand undisturbed for 10 minutes. The supernatant containing the hemolysed red blood cells is discarded. Saline solution is added and mixed with cells and the solution passed through a membrane filter (Keettel *et al*., 1974; Niwayama *et al*., 1976 and Yam *et al*., 1983).

Stains

Fundamentally 2 different methods of fixation and staining are used in FNA cytology. Air drying followed by staining with a hematological stains such as MGG, Wright, Giemsa Stain or Diffquik. Second one is alcohol fixation and staining with Papanicolaou or H&E. Both methods have their advantages and disadvantages. Air drying causes the cell to flatten on the glass surface. Therefore it appears larger than the cell fixed in ethanol. But nuclear enlargement and variation in nuclear size are exaggerated in wet fixed Papanicolaou stain and this is helpful in cytological diagnosis.

Difficulties can also occur with wet fixation particularly drying artifacts if samples are thick and highly cellular. However with both these methods, cells were lost either in the fixative solution or subsequently during staining.

In the past decade, FNAC has gained tremendous growth with many improvements in the technique. The new advanced techniques which has improved the efficacy of FNAC includes special stain techniques, immuno-cytochemistry, cell block preparation techniques, quantitative techniques like morphometry, object counting, flow cytometry, *in situ* hybridization technique and polymerase chain reaction.

In spite of development of new advanced ancillary techniques, FNAC has its own limitations. The diagnostic accuracy of FNAC depends on adequacy of sample, representativeness of the sample and good cyto-morphological detail without much artifactual distortion. Several studies had been done to reduce pit falls and improve the diagnostic accuracy of FNAC.

In 1958 Chandler Foot *et al*. experimented with the supra vital stains (Neutral red - Janus green) in sediments of effusion. The most frequent and disturbing obstacle to accurate cytological diagnosis of cancer in sediments of serous effusions is the fact that histiocytes and mesothelial cells may undergo misleading metaplastic change under certain circumstances (*eg.* congestive cardiac failure, cirrhosis) and are then readily mistaken for cancerous elements. This Neutral red - Janus green procedure which has been extensively studied by others (Simpson *et al*., 1922 and Sabin *et al*., 1924) offers a simple method for positively identifying histiocytes and leukocytes. Neutral red stains the granules of the histiocytes a brilliant orange red and janus green stains lymphocytes diffusely sky blue and colours the mitochondria. Mesothelial cells do not stain at all by this method. Their study established a diagnostic accuracy of 38.2% (13/34) by supra vital stains. This pilot study using supra vital stain wet mounts had its limitations as the authors (Chandler *et al*., loc cit 1958) themselves acknowledge that severe neoplastic cells could not take up these stains.

Other rapid stains used for wet sediment examination are thionine blue, methylene blue and toludine blue. Harris and Keebler (1976) suggested the use of these stains as a,

(i) control when setting up new cytopreparatory procedure.

(ii) check on the cellular preservation of the sample.

(iii) method of identifying highly positive samples so they may be stained separately.

(iv) check on existing cytopreparatory method.

(v) mean of estimating the cellularity of a sample.

Other cytopathologists recommended the use of these stains as a definitive diagnostic procedure for some fluid specimens such as urine, effusions and spinal fluid (Leopald G. Koss, 1979).

Ferreira *et al*. (1959) studied fresh material cytology using toludine blue. Toludine blue is a basic dye of thiazine group that selectively stains acidic tissue components like carboxylates, sulfates, phosphate radicals, DNA and RNA (Herlin *et al*., 1983 and Martin *et al*., 1998). Cancer cells contain quantitatively more DNA and RNA than normal epithelial cells and so toludine blue stains nuclei of

malignant cell well and give satisfactory nuclear details. Bennion *et al*. (1975) and Lepage *et al*. (1975) studied and demonstrated the greater affinity of tumor RNA for basic dyes like toludine blue.

Richart (1962) and Shedd (1965) studied *in vivo* staining of oral cancer using toludine blue. They concluded that neoplastic, cells stained intensely with toludine blue and non dysplastic epithelial cells fail to retain toludine blue stain. Malignant epithelium may contain intra cellular canals that are wider than normal epithelium and this is a factor that would enhance penetration of dye. Later Silverman *et al*. (1984) studied the usefulness of toludine blue in detection of oral precancerous and malignant lesions. Their result yielded the accuracy of toludine blue uptake 91%, false negatives were 2%. There were 30% false positives in benign lesions.

This *in vivo* staining technique using toludine blue were also studied in many tissues other than oral lesions. In 1976, Giler *et al*. experimented per oral intra gastric staining method using toludine blue in forty two cases with suspected neoplastic lesions of the stomach. Their study showed fifteen out of eighteen malignant lesions and one out of thirteen benign lesions were stained. The normal mucosa, areas of inflammation and most of the benign lesions appeared unchanged. They concluded that *in vivo* toludine blue staining prior to endoscopy might be of help in demonstrating minute malignant lesions of gastric mucosa and in differentiating benign and malignant ulcers of stomach.

Since toludine blue is a good nuclear stain, combination of toludine blue with other dye is used to improve cytomorphology by creating excellent contrast. This combination stain study was experimented by Henriques, (1972), where he used toludine blue and eosin as a stain for rapid cytodiagnosis. Later Vartanian *et al*. (1998) tried the efficacy of this combination stain. He used Leung stain which is a combination of toludine blue and alcian yellow for demonstration of Helicobacter pylori. Their study on the reliability of the Leung stain in endoscopic mucosal biopsy specimen revealed that it is cheapest, easiest to prepare and good choice as a standard for routine Helicobacter pylori staining.

Later a comparison study between different stain were experimented by Linda *et al*. (1978). Studies had been done to compare the efficacy of toludine blue stain as a supplementary staining with Grocotts modification of Gomori's stain for demonstration of Pneumocystis carini. Their study showed positive results in 67/78 (86%) of cases with toludine blue stain and 60/78 (77%) of cases with Gomoris stain alone and accuracy using both stains was 100%. They concluded that it is a rapid stain, accurate and highly constant.

Hympherys *et al*. (1996) did a pilot study comparing toludine blue and H&E staining of Basal cell carcinoma and Squamous cell carcinoma during mohs surgery. Their study showed that toludine blue revealed stromal changes associated with the presence of Squamous cell carcinoma and Basal cell carcinoma. H&E provided more prominent visibility of individual cell keratinisation and necrosis, which are common features seen in Squamous cell carcinoma.

In vivo and *vitro* gross staining comparison studies were done by David *et al*. (1989). They compared the efficacy of toludine blue and methylene blue as a visual marker for breast mass

localisation *in vivo* and *vitro*. Precise pre operative localization is important for reducing false negative results and decreasing the size of the biopsy specimen needed and resulting breast deformity. Their results suggested that toludine blue causes less discomfort and produces a more intense stain with smaller diffusion radius than methylene blue regardless of breast parenchymal pattern.

Adequacy of the sample is more important for cytological diagnosis. Inadequate and scanty sample pose a major problem in arriving at inconclusive diagnosis. Several authorities stated that immediate on site smear evaluation by cytopathologist optimizes diagnostic accuracy and minimizes the technique insufficiency rate. This favourable effect on FNA diagnostic accuracy is most pronounced for deep body seated lesions where FNA is guided by computed tomography, ultrasound, bronchoscopy and endoscopy. Several studies had been done on immediate assessment of cellularity using rapid stains to improve diagnostic accuracy.

Civardi *et al*. (1988) studied the value of rapid staining and immediate cellularity assessment of ultrasound guided fine needle aspirate samples. Their result yielded a sensitivity of 95.6%. They concluded that rapid evaluation of aspirated material can reduce the number of punctures needed per case resulting in less discomfort and reduced likelihood of complication for the patient.

Later Silverman *et al*. (1989) studied the accuracy of immediate interpretation of FNAC from various sites. He utilized Diff-quik as a rapid stain for immediate assessment. Diff-quik is a modified Wright stain (Marluce Bibbo, 1997). It provides good cellular detail and identifies stromal fragments by metachromasia. It includes 2 solutions. Solution I is buffered eosin Y. Solution II is a buffered solution of thiazine dyes methylene blue and azure A. Azure A undergoes slow constant oxidation to azure B which is the actual staining solution of the original Romanowsky method. Here air dried fixed smears were dipped for 5 seconds in solution I and II respectively. Then the slide was rinsed with water. The slide was allowed to dry or examined wet. Their study yielded a sensitivity of 96%. There were 14 false negative or falsely insufficient immediate interpretations and one false positive immediate diagnosis. They concluded that immediate assessment can,

(i) Determine whether an adequate specimen is present.

(ii) Render a specific preliminary diagnosis.

(iii) Guide further clinical investigations or treatment.

(iv) Determine whether ancillary studies are needed to make a more accurate or specific diagnosis from the FNA specimen.

Later the same study was done by Fabre *et al*. (1999) for immediate assessment of guided FNAC from deep seated masses using Diff-quik stain. Their study showed a rapid evaluation increases the diagnostic yield allowing near 100% in sensitivity, specificity and predictive value of positive cases.

Kusum Verma *et al*. (1991) studied the diagnostic accuracy of immediate interpretation of fine needle aspirates from various sites. But here they followed rapid MGG staining method as a rapid stain for their study. Air dried fixed smears were stained by applying may Grunwald stain and Giemsa stain (MGG) for one minute each respectively. Then the smears were air dried, examined and morphologic

diagnosis was rendered. The entire procedure took 10 - 15 minutes. Later the routine MGG staining was done on the same slide where May Grunwald stain and Giemsa stain was applied for 10 minutes and 15 minutes respectively. They compared this MGG staining and routine Papanicolaou staining and the results of immediate interpretation were compared with the final diagnosis and statistically analysed. Their result showed a sensitivity of 97%. The diagnostic accuracy of immediate interpretation of FNA smears found by them were comparable with 97.5% to 98.4% accuracy reported for frozen section diagnosis (Saltzstein *et al*., 1973 and Lessells *et al*., 1976, Rogers *et al*., 1987). Their study confirmed the utility of the test for rapid intra operative diagnosis and this technique proved to be a valuable tool and replaces the frozen section particularly when facilities for the latter are not available.

In 1992, Srivannaboon *et al*. studied and compared the diagnostic accuracy of toludine blue with that of Papanicolaou stain in non gynecologic cytology. In their study, a sensitivity of 95.3% and 96.9% was achieved by using toludine blue and Papanicolaou stain respectively. They found that the diagnostic accuracy of toludine blue stain approximates that of the Papanicolaou stain.

Mitotic index is a clinically important parameter in cancer pathology. Chieco *et al*. (1993) studied the use of toludine blue as a rapid stain to detect mitotic figures in formalin fixed paraffin embedded human and rat tissues. Here sections were stained at acid pH (3.5) with a 0.01% toludine blue solution after removal of RNA with hydro chloric acid or ribonuclease. This procedure stained mitotic figures much more intensely than other cellular structures.

Later the application and accuracy of intra operative immediate cytological assessment by rapid stains in place of frozen sections were studied. Chang *et al*. (1993) studied three hundred and five fresh specimens submitted for intra operative frozen stain for immediate intra operative cytological assessment. They experimented Liu's stain as a rapid stain. Liu's stain is one of the Romanowsky stain that take only 2 minutes. The accuracy of diagnosis was measured by comparison with final histological diagnosis. Sensitivity and specificity of intra operative cytology were 94.9% and 95.6% respectively. In combination of intra operative cytology and frozen section, sensitivity and specificity become 96% and 96.3% respectively. They concluded that Liu's stain is a simple, rapid, reliable staining method in intra operative cytological diagnosis. It is apparent that intra operative cytology is able to provide a useful adjunct to the frozen section diagnosis.

Yang *et al*. (1995) studied an alternative procedure for fine needle aspiration cytology. The objective of this study was to develop a Papanicolaou stain as fast as Diff-quik yet the cytomorphology as exquisite as that processed by thin prep for the optimal evaluation of fine needle aspirates. Satisfactory results were obtained after three modifications were made,

(i) Rehydration of air dried smears with normal saline.

(ii) Use of a 4% formaldehyde or 65% ethanol fixative.

(iii) Use of Richard Allen Hematoxylin 2 and cyto-stain.

The first modification restored the transparency of the cells and hemolysed red blood cells, the second modification reduced the time needed for proper fixation and staining from minutes to seconds

and the third modification simplified the procedure. This 90 second protocol yields a transparent polychromatic stain with crisp nuclear and cytoplasmic features. The cytomorphology processed by this protocol is atleast equal to, if not better than the quality of specimens prepared by thin prep and superior to those processed by the standard papanicolaou procedure. This ultra fast stain (Yang, loc cit 1995) can also be adapted for permanent FNA smears.

CT, endoscopic and ultra sonography guided FNAC is safe, rapid and cost effective method for securing a sample of abnormal tissue to diagnose and stage a variety of pathologic conditions in deep seated organs. The rate of false negative results is more dependant upon sampling failure and poor preparation of aspirated material than on interpretation error. Lachman *et al*. (1995) studied three hundred and forty one cases of image directed fine needle aspirates for onsite adequacy assessment (OSAA). The diagnostic accuracy before and after the implementation of this onsite adequacy assessment (OSAA) were compared. This study yielded a diagnostic sensitivity of 86% before OSAA and of 98% after OSAA.

Studies done by many authors on immediate assessment of FNAC from various sites proved the value of its diagnostic accuracy. Later this was concentrated on FNAC of particular tissue and studied. Stewart *et al*. (1996) studied the value of immediate assessment of cytology in FNAC of lung. They utilized Diff-quik as a rapid stain. The diagnostic accuracy was examined by review of clinical and radiological data in all patients. All malignant diagnoses were confirmed on clinical or pathological review and the diagnostic sensitivity was 96.6%. They concluded that immediate cytology assessment reduces the number of unsatisfactory and false negative lung FNAC. The complication rate is also minimized by decreasing the number of pleural punctures.

Tsou *et al*., (1997-98) experimented Riu's stain as a rapid stain for cytologic diagnosis of thyroid and liver tumors. Riu's stain is a Romanowsky type stain and has been in use in Taiwan over the past forty years. Their study showed a sensitivity of 93.5% and specificity of 100% for the detection of malignancy. They concluded that Riu's stain is a reliable quick stain in the diagnosis of lung and thyroid malignancy.

Later, Baloch *et al*. (2000) evaluated the combined impact of ultrasound guidance rapid on site evaluation of FNA specimen and different cytologic preparations (fresh and alcohol fixed smears, Millipore filter) and staining method (Diff-quik and Papanicolaou stain) on the diagnostic yield of thyroid FNA. A definite diagnosis could be made solely on the basis of air dried Diff-quik stained preparations in 65% cases, alcohol fixed papanicolaou stained smears in 68% cases and Millipore filter preparation in 91% cases. They concluded that ultra sound guided FNA combined with onsite evaluation and different cytologic preparation on significantly improve the diagnosite accuracy of thyroid FNA specimens.

One of the limitations of fine needle aspiration of thyroid is difficulty in distinguishing the follicular variant of papillary thyroid carcinoma from follicular neoplasms. By highlighting the "orphan-anne eyed" clear nuclei of the former, the ultra fast papanicolaou stain easily separates these two entities. In 2001, Yang *et al*., assessed 1135 ultrasound guided FNAs of thyroid. Of the 1127 satisfactory FNAs with 2-6 years clinical follow up, a false negative rate of 0% and a false positive rate

of 1.5% were obtained. Of the 169 surgical follow-ups with satisfactory FNAs, a sensitivity of 100%, specificity of 66.7%, positive predictive value of 87.4%, negative predictive value of 100% and global accuracy of 89.9% were obtained.

Shirley *et al.* (2003) again studied the utility of rapid staining of FNAC. Sensitivity and specificity values were similar for rapid and routine stained slides and ranged from 80-100%. Their study concluded that rapid staining of cytological smears is a useful adjunct to the evaluation of aspirated material, improving adequacy rates and over all performance of the FNA service and should also result in significant savings in time and cost to patients.

Now rapid staining of FNAs is an accepted procedure for evaluation of adequacy and rapid diagnosis. Several studies had been conducted on the rapid staining techniques so far using stains such as Diff quik, rapid MGG, Liu's stain, Riu's stain, ultra fast pap. However those stains are imported and expensive.

Recently Joy, M.P. *et al*. (2003) examined toludine blue as an alternative rapid stain. It is an inexpensive stain, and easily available. Although toludine blue has been used in evaluation of touch imprint, frozen sections and in squash preparation of central nervous system tumors (Dusmez *et al*., 2001), there were no pervious reports using toludine blue for rapid diagnosis of ultrasound guided fine needle aspirates. They studied the reliability of touldine blue stain as a rapid stain for quick diagnosis in ultra round guided aspiration cytology. Here smears were air dried and dipped in a Coplin jar containing freshly filtered staining solution for one minute. Then rinsed in tap water. Wet mounted slides were examined under the microscope. They observed that cytoplasmic, nuclear details were well appreciated in toludine blue stained smear permitting rapid diagnosis. The sensitivity of their study for malignant/suspicious for malignancy was 98.54%. Sensitivity and specificity for an inflammatory condition was 100%. They concluded that toludine blue staining is not only a reliable method for rapid staining and diagnosis, it also permits preservation of cytological material by destaining and restaining with permanent stains.

Destaining was done by putting the smear in 95% alcohol for ten minutes and then it was used for restaining with MGG.

Wet mount studies were done previously in effusion fluids, urine cytology using toludine blue for rapid diagnosis (Zuher, 1985).

Various studies on toludine blue reveal that, toludine blue is a good nuclear stain and a reliable rapid stain. In all above studies smeared samples are studied for immediate assessment of cytomorphology and rapid diagnosis. One problem in FNAC is morphological distortion due to drying and smearing artifact. This may be avoided in the great extent by using wet mount techniques.

Materials and Methods

The study materials include fine needle aspirates, obtained from lesions of breast, lymphnode, thyroid, salivary gland, cysts, soft tissue masses and cavity fluids in the cytology out-patients department of Tirunelveli Medical College Hospital. A total of 197 cases were chosen for study during the period of Jan. 2004 to Dec. 2005.

Materials

(i) Needles – 23 G. 20-50 mm needle for superficial lesion.
(ii) 23-22 G – 90 mm needle for guided FNAC.
(iii) Syringes, spirit, cotton, slides, coverslips, test tubes, centrifuge.
(iv) Standard Hematoxylin and Eosin stains (H & E)
(v) Toludine blue 0.5%
(vi) Eosin 0.5%
(vii) A fixative 70-90% ethanol for H & E

Gloves and masks are required as a precautionary measure.

Preparation of rapid supravital stain

a) Toludine blue

i. Toludine blue 0.5 G
ii. 95% alcohol 20 ml
iii. Distilled water 80 ml

b) Aquous eosin

i. Eosin Y 0.5 G
ii. Distilled water 100 ml

Method

Toludine blue was dissolved in alcohol. Then distilled water was added and filtered through the Whatman No. 1 filter paper. Solution was stored in refrigerator.

Aquous eosin stain was prepared by dissolving 0.5 gm of eosin in distilled water. Then the solution was filtered using Whatman No. 1 filter paper and stored.

For each case atleast two conventional H & E stained smear and two toludine blue/eosin stained wet mount were prepared.

(i) Conventional fixed smear preparation using H & E

The above procedure was done conventionally in all cases (Svante R. Orelle *et al.,* 1999).

(ii) Wet mount preparation using toludine blue - eosin stain

(i) Drop of fine needle aspirate was expressed in centre of slide.

(ii) In case of body cavity fluid cytology drop of fluid was placed in the centre of slide if fluid was turbid or the fluid was centrifuged at a speed of 1500 rpm/mt for 10 minutes. The supernatant fluid was discarded. Then a drop of sediment was placed in the centre of slide.

(iii) A drop of toludine blue stain was placed next to cell sample.

(iv) 2 drops were mixed together with a needle. A drop of eosin solution was placed next to cell stain mixture. The sample was mixed well.

(v) Small 22 x 30 mm coverslip was placed over the sample.

(vi) The slide was examined under microscope and the sample was evaluated immediately.

(iii) Alternative method of preparation of wet mount

This method was tried whenever aspirates were very scanty and adhered to hub of the needle. Under such condition it was very difficult to express the aspirate over the slides. Hence,

(i) A few drops of toludine blue stain was aspirated using the same syringe and needle and rinsed.

(ii) Then the stain mixed material was expressed in the centre of slide.

(iii) A drop of eosin solution was placed next to cell stain mixture and mixed well.

(iv) Cover slip was placed over it.

(v) Wet mount was examined under microscope and cytomorpholoty was observed.

In cases where slides have to be preserved for few hours, the coverglass over the sample was sealed off by applying melted Vaseline or DPX. This sealing helps to retain cytomorphology for a period of three hours without morphological distortion and it also prevents quick drying of wet mount.

RESULTS AND OBSERVATION

DISTRIBUTION OF LESIONS (Table Ia.)

Site	No. of cases	Percentage (%)
1. Lymph node	62	31.5
2. Thyroid	49	24.9
3. Breast	41	20.8
4. Soft tissues	14	7.1
5. Fluid cytology	14	7.1
6. Salivary gland	7	3.5
7. Bone	5	2.5
8. Deep seated lesions	5	2.5
Total	**197**	**100**

DIAGNOSTIC ACCURACY (Table Ib.)

	H&E	TB/E and H&E Combined
Diagnostic accuracy	87%	97.4%
Erroneous result	13%	2.6%

LYMPH NODE LESIONS (Table IIa.)

Nature of lesions	Total no of cases and %	No & % of positive cases		
		TB/E	H&E	Biopsy Confirmation
1. Reactive node	13 (21%)	13 (100%)	12 (92.3%)	10 (77%)
2. Suppurative node	2 (3.2%)	2 (100%)	2 (100%)	-
3. Granulamatous lymphadenitis	26 (42%)	24 (92.3%)	21 (80.8%)	26 (100%)
4. Metastatic deposit	18	18	16	18

	(29%)	(100%)	(88.9%)	(100%)
5. Lymphomas	3 (4.8%)	2 (66.7%)	2 (66.7%)	3 (100%)
Total	62 (100%)	59 (95.2%)	53 (85.5%)	57 (91.9%)

Out of sixty two cases, TB/E (Toludine blue/Eosin) was more useful in optimizing the accuracy of conventional H&E in six cases.

DIAGNOSTIC ACCURACY (Table IIb.)

	H&E	**TB/E and H&E Combined**
Diagnostic accuracy	85.96%	98.2%
Erroneous result	14%	1.8%

THYROID LESIONS (Table IIIa.)

Nature of lesions	Total no of cases and %	No & % of positive cases		
		TB/E	H&E	Biopsy Confirmation
1. Nodular colloid goiter	22 (44.9%)	20 (90.9%)	18 (81.8%)	22 (100%)
2. Diffuse adenomatous goitre	17 (34.7%)	17 (100%)	17 (100%)	16 (94.1%)
3. Hashimatos Thyroiditis	6 (12.2%)	2 (33.3%)	6 (100%)	6 (100%)
4. Malignancy	4 (8.16%)	4 (100%)	4 (100%)	4 (100%)
Total	49 (100%)	43 (87.8%)	45 (91.8%)	48 (98%)

Out of forty nine cases, diagnostic accuracy of H&E was more useful than TB/E (Toludine blue/Eosin) stain study in two cases.

DIAGNOSTIC ACCURACY (Table IIIb.)

	H&E	TB/E and H&E Combined
Diagnostic accuracy	91.7%	95.8%
Erroneous result	8.3%	4.2%

BREAST LESIONS (Table IVa.)

Nature of lesions	Total no of cases and %	No & % of positive cases		
		TB/E	H&E	Biopsy Confirmation
1. Fibroadenoma	4 (9.75%)	4 (100%)	4 (100%)	4 (100%)
2. Fibrocystic disease	15 (36.6%)	13 (86.7%)	12 (80%)	12 (80%)
3. Inflammatory disease	2 (4.9%)	2 (100%)	2 (100%)	1 (50%)
4. Phyllodes tumor	3 (7.3%)	3 (100%)	2 (66.7%)	3 (100%)
5. Malignancy	17 (41.5%)	16 (94.1%)	15 (88.2%)	17 (100%)
Total	41 (100%)	38 (92.7%)	35 (85.4%)	37 (90.2%)

Out of forty one cases, TB/E (Toludine blue/Eosin) was more useful in optimizing the accuracy of conventional H&E in three cases.

DIAGNOSTIC ACCURACY (Table IVb.)

	H&E	TB/E and H & E Combined
Diagnostic accuracy	91.9%	100%
Erroneous result	8.1%	0%

SUBCUTIS AND SOFT TISSUE LESIONS (Table Va.)

Nature of lesions	Total no of cases and %	No & % of positive cases		
		TB/E	H&E	Biopsy Confirmation

1. Cystic lesions	6 (42.9%)	6 (100%)	5 (83.3%)	6 (100%)
2. Benign	5 (35.7%)	5 (100%)	4 (80%)	5 (100%)
3. Malignancy	3 (21.4%)	3 (100%)	2 (66.7%)	3 (100%)
Total	14 (100%)	14 (100%)	11 (78.6%)	14 (100%)

Out of fourteen cases, TB/E (Toludine blue/Eosin) was useful in optimizing the accuracy of conventional H&E in three cases.

DIAGNOSTIC ACCURACY (Table Vb.)

	H&E	**TB/E and H&E Combined**
Diagnostic accuracy	78.6%	100%
Erroneous result	21.4%	0%

FLUID CYTOLOGY (Table VIa.)

Nature of lesions	Total no of cases and %	No & % of positive cases	
		TB/E	H&E
1. Inflammatory lesions	8 (57.1%)	8 (100%)	6 (75%)
2. Malignancy	6 (42.9%)	6 (100%)	4 (66.7%)
Total	14 (100%)	14 (100%)	10 (71.4%)

Out of fourteen cases, TB/E (Toludine blue/Eosin) was useful in optimizing the accuracy of conventional H&E in four cases.

DIAGNOSTIC ACCURACY (Table VIb.)

	H&E	TB/E and H&E Combined
Diagnostic accuracy	71.4%	100%
Erroneous result	28.6%	0%

SALIVARY GLAND LESIONS (Table VIIa.)

Nature of lesions	Total no of cases and %	No & % of positive cases		
		TB/E	H&E	Biopsy Confirmation
1. Benign	5	5	5	5

	(71.4%)	(100%)	(100%)	(100%)
2. Malignancy	2 (28.6%)	1 (50%)	1 (50%)	2 (100%)
Total	7 (100%)	6 (85.7%)	6 (85.7%)	7 (100%)

Accuracy of TB/E (Toludine blue/Eosin) and H&E studies were equal and both missed one case of malignant salivary gland lesion.

DIAGNOSTIC ACCURACY (Table VIIb.)

	H&E	**TB/E and H&E Combined**
Diagnostic accuracy	85.7%	85.7%
Erroneous result	14.3%	14.3%

BONE LESIONS (Table VIIIa.)

Nature of lesions	Total no of cases and %	No & % of positive cases		
		TB/E	H&E	Biopsy Confirmation
1. Benign	2 (40%)	2 (100%)	2 (100%)	2 (100%)
2. Malignancy	3 (60%)	3 (100%)	2 (66.7%)	3 (100%)
Total	5 (100%)	5 (100%)	4 (80%)	5 (100%)

Out of five cases, TB/E (Toludine blue/Eosin) was more useful in optimizing the accuracy of H&E study in one case.

DIAGNOSTIC ACCURACY (Table VIIIb.)

	H&E	TB/E and H&E Combined
Diagnostic accuracy	80%	100%
Erroneous result	20%	0%

DEEP VISCERAL LESION (Table IXa.)

Nature of lesions	Total no of cases and %	No and % of positive cases	
		TB/E	H&E
1. Lung malignancy	4 (80%)	4 (100%)	3 (75%)
2. Mediastinum malignancy	1 (20%)	1 (100%)	-
Total	5 (100%)	5 (100%)	3 (60%)

Out of five cases, TB/E (Toludine blue/Eosin) was useful in optimizing the accuracy of H&E in two cases.

DIAGNOSTIC ACCURACY (Table IXb.)

	H&E	TB/E and H&E Combined
Diagnostic accuracy	60%	100%
Erroneous result	40%	0%

OBSERVATIONS

Cytomorphological analysis and diagnostic accuracy of toludine blue/eosin stained wet mount preparation of fine needle aspirates were studied and compared with that of conventional H&E stained fixed smear. We performed this study on 197 cases of fine needle aspirates obtained from various sites, which includes lymph nodes, breast, soft tissues, body fluids, thyroid, salivary gland, bone and deep viscera.

Distribution of lesions

Total number and percentage of cases we studied are given in Table I. From the table it is observed that accuracy of FNAC using H&E alone is 87% and it is improved to 97.4% when we combined supravital stained wet mount study. The erroneous result also decreased from 13% to 2.6% (Table I).

ψ^2 value is 4 which is more than the table value 1.96. So P value is less than 0.05, which is statistically significant.

Lymph node aspiration

Of the 62 cases of lymph node aspirates, rapid stain yielded a diagnostic accuracy of 95.2% where as H&E alone gave 85.5% accuracy. We received biopsy specimen for 57 cases since for reactive and suppurative lesions clinicians were convinced with FNAC report and did not ask histopathological confirmation. The overall diagnostic accuracy in lymph node aspirates using H&E alone is 85.96%. It is 98.2% when we combined rapid staining of wet mount as a supplementary procedure (Table II).

ψ^2 value is 2.51 which is more than table value 1.96. So P value is less than 0.05, which is statistically significant.

Thyroid aspiration

Supravital stained wet mount of thyroid aspirates yielded 87.8% accuracy whereas our conventional H&E gave 91.8% accuracy. The decreased accuracy in supravital stained study is due to problems in identifying Hurthle cells, which is well appreciated by H&E study. Still the diagnostic accuracy of H&E is improved from 91.8% to 95.8% in our study by combining wet mount analysis (Table III).

Breast aspiration

Of the 41 cases of breast aspirates, rapid stain study yielded a diagnostic

accuracy of 92.7% where as conventional H&E gave 85.4% accuracy. We received biopsy specimen for 36 cases since for inflammatory lesion and for three cases of fibrocystic disease they did not ask biopsy confirmation. Biopsy reports confirmed the accuracy of rapid stain study. The overall diagnostic accuracy using H&E alone is 91.6% whereas it is 100% when we combined rapid stain study (Table IV).

ψ^2 value is 1.98 which is more than the table value 1.96. So P value is less than 0.05 which signifies the test.

Soft tissue aspiration

Rapid stain study yielded 100% accuracy in the study of 14 cases of soft tissue aspirates whereas conventional H&E gave 78.6% accuracy. This 100% accuracy of rapid stain is confirmed by histopathological study also. So overall diagnostic accuracy is improved to 100% when rapid stain study is supplemented with conventional H&E (Table V).

ψ^2 value is 2.05 which is more than table value 1.96. So P value is less than 0.05, which is statistically significant.

Body fluid cytology

Of the 14 cases of fluid cytology, rapid stain study gave 100% accuracy whereas H&E alone study yielded 71.4% accuracy. The drawback of fluid cytology using H&E stained fixed smear is loss of cell sample during fixation and staining resulted in inconclusive diagnosis. This is minimized in wet mount study. Here rapid diagnosis is confirmed by clinical and radiological follow up. The overall diagnostic accuracy is improved to 100% by combining wet mount study with conventional H&E (**Table VI**).

ψ^2 is 2.31 which is more than the table value 1.96. So P value is less than 0.05 which signifies the test.

Salivary gland aspiration

Of the 7 cases of salivary gland aspirates, both rapid stain and H&E study yielded 85.7% accuracy. We missed one case of malignant salivary gland lesion both with TB and H&E which is later confirmed as malignancy by histopathological examination (Table VII).

Bone aspiration

Here rapid stain study yielded 100% accuracy in 5 cases of bone aspirates whereas H&E study gave 80% accuracy. Rapid diagnosis is confirmed by histopathological examination also. The diagnostic accuracy of H&E is improved to 100% by doing rapid stain study simultaneously (**Table VIII**).

Deep visceral aspiration

Of the 5 cases of aspirates obtained from deep viscera, rapid stain gave 100% accuracy. H&E yielded only 60% accuracy due to inconclusive diagnosis as a result of scanty cellularity and masking of cytomorphology by necrotic material. Here also rapid diagnosis is confirmed by clinical and radiological follow up. The overall diagnostic accuracy of H&E is improved to 100% by simultaneously doing rapid staining of wet mount film study (**Table IX**).

Cytomorphology

Lymph node

Aspirate from Reactive node lesions showed centroblast - cell with scanty blue distinct cytoplasm, round nucleus with granular chromatin and 1-2 nucleolus. Immunoblasts - large cell with moderate blue cytoplasm, large round nucleus with granular chromatin and single nucleolus.

Suppurative lesions showed clusters of neutrophils and pale pink granular necrotic material, karyorrhectic nuclei. Sometime mobile bacteria is also seen.

Granulomatous lesions showed clusters of epithelioid cells – elongated cell with indistinct cytoplasmic membrane, pale blue cytoplasm, vesicular nucleus with prominent nucleoli and polymorphous population of lymphocytes, multinucleated giant cells and few cyst macrophages with purplish pink granular necrotic material in the background (**Fig. No. 1**).

Metastatic adeno carcinomatous lesions showed pleomorphic cells with scanty blue cytoplasm, large round, oval, irregular hyperchromatic nucleus with inconspicuous nucleoli.

Metastatic squamous cell carcinomatous lesions showed pleomorphic cells with scanty to moderate pink cytoplasm and large irregular hyperchromatic nucleus with inconspicuous nucleoli (**Fig. No. 2**).

Lymphoproliferative lesions showed monomorphic population of large cells with scanty blue cytoplasm, large round nucleus with granular chromatin and prominent nucleoli (**Fig. No. 3**).

Thyroid

Colloid goiter showed thick colloid as patchy blue colored material and thin colloid as purplish pink colored granular material. Follicular cells were seen as round mild pleomorphic cell with scanty cytoplasm and mild nuclear anisokaryosis.

Aspirate from papillary carcinoma lesion showed papillary sheets of monomorphic cells with moderate pale pink cytoplasm, large round uniform nucleus with prominent small basophilic nucleolus and some nucleus with inclusion (**Fig. No. 4 and 5**).

Aspirate from anaplastic carcinoma showed pleomorphic cell with scanty pink cytoplasm, large irregular hyperchromatic nucleus (**Fig. No. 6**).

Breast

Aspirate from benign fibroadenoma lesions showed uniform round to oval cells with scanty to moderate pale pink cytoplasm, round to oval uniform nucleus with granular chromatin and many bare ovoid nuclei (**Fig. No. 7**).

Fibrocystic lesions showed benign duct epithelial cell, cyst macrophages-large round cell with vacuolated cytoplasm and round nucleus, eosinophilic secretions and few spindle cells entrapped within fat cell clusters. Aspirate from fat necrosis slowed many fat globules entangled in pale pink granular necrotic material and few macrophages.

Malignant lesions showed pleomorphic cells with scant to moderate blue cytoplasm, large darkly stained nucleus with smudged chromatin and inconspicuous nucleoli and necrotic material in the background (**Fig. No. 8 and 9**).

Phyllodes tumor aspirates showed cluster of duct epithelial cells round to oval cells with scanty cytoplasm, round uniform nucleus with single small nucleoli, and cluster of stromal cell-oval to spindle cells with indistinct cytoplasmic membrane, scanty pink cytoplasm mild anisokaryotic nucleus with granular chromatin and small nucleolus (**Fig. No. 10 and 11**).

Soft tissue

Cystic lesions

Thick pus like fluid from bronchial cyst lesions showed sqaumous epithelial cells with few neutrophils and lymphocytes in the background.

Lymph cyst lesions showed clear fluid aspirate and scattered population of uniform lymphocytes.

Thyroglossal cyst lesions slowed scattered thyroid follicular cells with purplish pink granular colloid in the background (**Fig. No. 12**).

Epithelial inclusion cyst lesions showed polyhedral purple colored anucleated squames (**Fig. No. 13**).

Benign lesions

Aspirate from lipoma showed polyhedral to large round fat cells.

Angiomatous lesions showed full of pale pink RBC.

Malignant lesions showed, spindle, polyhedral, pleomorphic cell with abundant pale pink cytoplasm, large spindle to round nucleus with hyperchromatism and invisible nucleolus, some cells showed binucleation (**Fig. No. 14**).

Fluid cytology

Fluid from reactive effusion showed round mesothelial cells with distinct cell membrane, moderate pink cytoplasm, large round nucleus some binucleate cells, some signet ring cells with peripherally pushed round nucleus by large vacuole in the cytoplasm (**Fig. No. 15**).

Fluid from malignant effusion showed pleomorphic cell with scanty pale pink cytoplasm and large irregular hyperchromatic nucleus.

Salivary gland

Benign lesions showed round to oval cells with distinct cell membrane, moderate pink cytoplasm, plump ovoid nucleus with granular chromatin and uniform spindle cells.

Malignant lesions showed sheets of pleomorphic cells with scanty pink cytoplasm, large anisokaryotic hyper chromatic nucleus.

Bone

Giant cell lesion showed plenty of large cells with moderate pink cytoplasm and more than five uniform nuclei.

Malignant lesion showed clusters of spindle cells with scant to moderate blue cytoplasm, large oval to spindle plump anisokaryotic nuclei and pinkish purple osteiod material.

Deep visceral lesion

Malignant lesion from Lung showed pleomorphic cells with scanty pink cytoplasm, large anisokaryotic hyperchromatic nuclei.

Aspirate from mediastinal teratoma showed eosinophilic keratin, many degenerated epithelial nuclei and squamous cells in granular necrotic background.

discussion

Chandler Foot *et al.* loc cit (1958), Silverman *et al.* loc cit (1989) kusum Verma *et al.* loc cit (1991), Chang *et al.* loc cit (1993), Yang *et al.* loc cit (1995), Tsou *et al.* loc cit (1997) experimented various rapid stains such as Neutral red – Janus green, Diff-quik, Rapid MGG, Liu's stain, Ultra fast pap, Riu's stain respectively for immediate diagnosis.

This work was inspired by the earlier work of Joy, M.P. *et al.* loc cit (2003) where they applied toludine blue as a rapid stain for quick diagnosis of ultrasound guided aspiration cytology.

In our study a combination of toludine blue/eosin is used as supra vital wet mount stain which yielded a good diagnostic accuracy of 97.4%. The above authors applied this rapid stain for immediate assessment of cytology. However our study is aimed at improving the diagnostic accuracy of conventional H&E stained fixed smear study with additional information from toludine blue/eosin stained wet mount study. Here we have assessed the reliability of this rapid staining of wet mount aspirate, which has not been previously reported.

Caya *et al.* (1984) reported that false negative reports were resulted from unrepresentative aspirates. False negative aspirates may include normal or reactive elements but necrotic material is an additional source of error (Winning *et al.,* 1986). This problem of sampling error cannot be eliminated entirely in FNAC but it is found reduced by rapid cytology assessment. This sampling error is reduced in our study by simultaneously doing rapid wet mount study. One problem in FNAC diagnosis is lack of adequate sample or unsatisfactory specimen in some cases. The problem of scanty cellularity occurs in tissues with cystic degeneration and tissues with extreme desmoplasia. Cagle *et al.* (1993) reported that inadequate sampling was solely responsible for 10% false negative report in Lung FNAC. In our study the needle and hub are rinsed with toludine blue stain, which effectively washes all the cells collected in the lumen yielding an improved cellularity.

Degenerated cells and neoplastic cells are more fragile and distorted easily during smearing which created confusion in diagnosis. Trapping of cells within fibrin meshwork also distorted the morphology of cell. Since cytomorphology forms the basis for the cytodiagnosis, artifactual morphological distortion influences the diagnostic accuracy of FNAC. This smearing artifact is avoided in our study since we are using wet mount preparations. More over, this wet mount study also gave an additional advantage of appreciating cells in three-dimensional view. So cytomorphology is

well appreciated and enabling us to arrive an accurate diagnosis.

Loss of cell sample during fixation in alcohol and subsequent staining process also pose a major problem in arriving inconclusive diagnosis (Svante R. Orelle *et al.,* loc cit 1999). This is especially true in case of sampling of fluids. It is avoided in our study by doing wet mount cytological examination. Here cells are examined without fixation by supra vital staining. So there is no loss of cell sample and yields sufficient cellularity to render a rapid diagnosis.

One of the most important features in cytodiagnosis is the morphology of the nucleus (Svante R. Orelle *et al.,* loc cit 1999). The advantage of this supravital stain is that the cell structure is well preserved with toludine blue stain (David *et al.,* loc cit 1989). Toludine blue has a high affinity for DNA and hence absorbed rapidly into the nucleus. As the dysplastic and anaplastic cells contain more nucleic acid, the nuclear stains of tumor cells are very prominent with toludine blue (Martin *et al.,* 1997). In our study we have found excellent nuclear detail provided by toludine blue enabling an accurate diagnosis. The diagnosis of malignancy is confirmed in all our patients by pathological, clinical, radiological follow up.

The study of morphology of individual cell is on great focus in our study since individual cell morphology varies in air dried and wet smear preparation and wet mount. Cytomorphology observed in our study is compared with that described by Svante R. Orelle *et al.* loc cit (1999). The nucleus of epithelioid cell in granulamatous lymphadenitis is elongated and resembles sole of shoe with inconspicuous nucleoli (Svante R. Orelle *et al.,* loc cit 1999). In our study the nucleus of epthelioid cells appeared to be elongated with distinct nucleolus (**Fig. No. 1**).

Metastatic malignancy of lymphnode shows foreign cells amongst normal/reactive lymphoid cells and cytological criteria of malignancy. Cytologic criteria for malignancy is nuclear enlargement, pleomorphism, irregular coarse chromatin with prominent nucleoli (Svante R. Orelle *et al.,* loc cit 1999). In our study matastatic carcinomatous deposit of lymphnode showed pleomorphic cells with scanty to moderate pink cytoplasm and large irregular nucleus with smudged dark chromatin and inconspicuous nucleoli (**Fig No. 2**). This hyperchromatic nature of malignant nucleus is due to increased toludine blue dye uptake as a result of increased DNA, RNA content of the malignant nucleus (Martin *et al.,* loc cit 1997).

Cytological study of malignant lymphoma often gives a monotonous population of lymphoid cells with scanty cytoplasm, round nuclei with multiple small nucleoli and variable proportion of indented or even multilobated nuclei (Svante R. Orelle *et al.,* loc cit 1999). In our study lymphoma aspirates showed a monomorphic population of large cells with scanty blue cytoplasm, large round nuclei with granular chromatin and prominent one to three nucleoli (**Fig No. 3**).

Conventional H/E stained fibroadenoma aspirates show large branching monolayered sheets of uniform epithelial cells and numerous single bare nuclei of benign type and fragments of fibromyxoid stroma (Svante R. Orelle *et al.,* loc cit 1999). In our study sheets of uniform round to oval cells with scanty to moderate cytoplasm, round to oval uniform nuclei with granular chromatin and many bare

ovoid nuclei were observed. (**Fig No. 7**)

Ductal carcinoma of breast aspirates are characterised by irregular clusters of atypical cells with nucleomegaly and irregularity with prominent nucleoli. There are single cells with intact cytoplasm and necrosis in the background. (Svante R. Orelle *et al.,* loc cit 1999). In our study Ductal carcinoma aspirates showed pleomorphic cells with scanty to moderate cytoplasm, large darkly stained nucleus with smudged chromatin and inconspicuous nucleoli. Some times a necrotic material in the background was observed.

Cohesive fragments of highly cellular stroma composed of spindle cells with nuclear atypia or atypical bare spindle nuclei in the background along with benign sheets of epithelial cells are highly suggestive of phyllodes tumor (Svante R. Orelle *et al.,* loc cit 1999). In our study cluster of duct epithelial cells-round to oval cells with scanty cytoplasm, round uniform nuclei with single small nucleoli and cluster of stromal cells-oval to spindle cells with indistinct cytoplasmic membrane, scanty pink cytoplasm, mild anisokaryotic nuclei with granular chromatin and small nucleoli are characteristic (**Fig No. 10 and 11**).

Epidermal cyst aspirates show debris with anucleate squames and mature squamous epithelial cells (Svante R. Orelle *et al.,* loc cit 1999). In our study epidermal cyst aspirates showed polyhedral purple coloured anucleate squames (**Fig No. 13).**

Aspirates from Malignant fibro histiocytic tumor often show atypical spindle cells with elongated nuclei, marked nuclear pleomorphism, multinucleated tumor giant cells and bizarre nuclei with coarse irregular chromatin. Nucleoli is variable but often prominent. (Svante R. Orelle *et al.,* loc cit 1999). In our study Toludine blue/Eosin stained wet mount showed spindle, polyhedral pleomorphic cells with pale pink cytoplasm, large round to spindle hyperchromatic nuclei and invisible nucleoli and some cells with binucleation (**Fig No. 14**).

Reactive effusion smears show mesothelial cells with scanty to moderate cytoplasm and sharp cytoplasmic membrane, central or eccentric nuclei with coarse clumped chromatin, regular nuclear membrane and multinucleation in the background of other leukocytes, cellular debris (Zuher loc cit., 1985). Toludine blue/Eosin stained wet mounts showed round mesothelial cells with distinct cell membrane, moderate pink cytoplasm, large round normochromic nuclei, some binucleated cells, and reactive mesoepithelial cells with peripherally pushed round normochromic nuclei by large vacuole in the cytoplasm and few macrophages (**Fig No. 15**).

Malignant effusion in case of squamous cell carcinoma shows large amount of blood, inflammatory cells, protein deposit and malignant squamous cells which are vary in size and shape with hyperchromatic nuclei (Zuher loc cit., 1985). In our study malignant squamous cells appeared pleomorphic with scanty pink cytoplasm and large irregular hyperchromatic nuclei in the inflammatory cell background. Malignant effusion in case of adeno carcinoma shows acini, papillary sheets of pleomorphic cells with scanty to moderate to cytoplasm, irregular enlarged nuclei with prominent nucleoli (Zuher loc cit., 1985). In our study it shows acini, papillary sheets of pleomorphic cells with

scanty cytoplasm, large irregular hyperchromatic nuclei with inconspicuous nucleoli.

Colloid stains blue to purple and form thin membrane like coat often with folds and cracks (Svante R. Orelle *et al.,* loc cit 1999). In our study thick colloid stains as patchy dark blue color material and thin colloid stains as granular purple material (**Fig. No. 12**).

Smear from papillary carcinoma of thyroid shows sheets of cell with enlarged ovoid nucleus, granular chromatin and prominent nucleoli with cytoplasmic inclusions and nuclear grooves (Svante R. Orelle *et al.,* loc cit 1999). In our study papillary sheets of follicular cells showed nucleus with small prominent basophilic nucleoli and some with nuclear inclusion (**Fig No. 5**).

However we found that tumor cell with definite cytoplasmic criteria for diagnosis do not have this similar feature in toludine blue/eosin staining. This problem is noted in our study particularly in identifying Hurthle cells where the cytoplasmic granules could not be identified giving a low sensitively of 33.3% for Hashimatos thyroiditis. Moreover here cells are examined in fresh state so all the cells appeared larger than those in H&E fixed smear. Hence the lymphocytes were falsely interpreted as naked follicular epithelial cells.

Based on the earlier work of Chang *et al.* loc cit (1993) we have tried the reliability of this rapid staining of wet mount for intra operative cyto diagnosis in 2 cases of breast lesions. The results were good and confirmed by histopathological examination also.

The results of our study of rapid cytodiagnosis are comparable with those of the earlier works done by many authors. Chandler foot *et al.,* loc cit (1958) had a diagnostic accuracy of 80% using Neutral red Janus green as rapid stain. Silver men *et al.* loc cit (1989) got an accuracy of 96% by using Diff-quik as rapid stain. Kusum verma *et al.* loc cit (1991) obtained an accuracy of 97% where they used rapid MGG stain. Chang *et al.* loc cit (1993) showed an accuracy of 94.9% in their study using Liu's stain. The study of Tsou *et al.* loc cit (1997) yielded an accuracy of 93.5% where they used Riu's stain for rapid cytodiagnosis. Recently Joy, M.P. *et al.* loc cit (2003) studied the reliability of toludine blue stain and they got the sensitivity of 98.54%.

Our results agree with Joy, M.P. *et al.* (2003) where they used toludine blue as rapid stain and found increased sensitivity when compared to other rapid staining technique done by above authors. Our work argues well for projecting toludine blue/eosin stain as a supplementary staining procedure for conventional H&E. The diagnostic accuracy of H&E in our study is 87%. But the overall diagnostic accuracy after combining toludine blue/eosin with conventional H&E is 97.4%, which is 10.4% more than the sensitivity given by conventional FNAC alone.

This work highlighted the usefulness of rapid staining technique using toludine blue/eosin in FNAC's. It also indicates an increase in the sensitivity of cytodiagnosis.

conclusion

The role of immediate assessment of fine needle aspirate is well established in the literature for many years. However this practice has still not attracted much attention in our country mainly due to non-availability of trained personnel who can perform the spot assessment. Moreover the preferred rapid stain in the western parts of the world is Diff-quik an imported proprietary preparation which is expensive and difficult to procure. So based on the work done by Joy, M.P. *et al.* (2003) we have standardised a much simpler alternative in the form of rapid toludine blue/eosin staining which is found to be cost effective.

Wet mount study of FNAC improves the diagnostic accuracy by minimizing the smearing and drying artifact, loss of cell sample during fixation and staining (Svante R. Orelle *et al.* loc cit 1999) which influences the diagnostic accuracy. Moreover it is established that three-dimensional view of cells are well appreciated in this wet mount study. Individual cell morphology is well defined in our study and is compared with cytomorphology of H&E stained smear. The accuracy of toludine blue/eosin stained wet mount study is assessed by confirming the rapid diagnoses with final diagnosis by conventional FNAC using H&E, histopathological examination and clinical follow up. The reliability of this wet mount study is also compared with works done by various authors and is proved to be accurate. Our study results yielded a good diagnostic accuracy of 97.4% by combining rapid stain as a supplementary procedure for conventional H&E. The decreased sensitivity of H&E alone due to inadequate cellularity, loss of cell sample during fixation and staining and artifactual morphological distortion, is overcome by supplementary wet mount study and that yielded high accuracy rate.

This study concludes that the supravital stained rapid wet mount FNAC is useful as,

i) A simple reliable, cost effective rapid staining method.

ii) It helps to obtain sufficient cellularity in less cellular fibrotic lesions.

iii) It is also used to assess adequacy of sample especially for deep seated lesions and minimize false negative results.

iv) The cytomorphology is well appreciated in wet mount study.

v) It can be used for intra operative cytodiagnosis as an adjunct to frozen section diagnosis.

vi) It improves the diagnostic accuracy of conventional FNAC's stained with H&E.

vii) It can be routinely undertaken as a supplementary procedure for conventional H&E.

This work gives a new dimension to the art of FNAC and also opens a new door for further researches in this regard.

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